APPLICATION FOR PATENT

INVENTORS: SANJAY KUMAR, LAKHVIR LAL, PARAMVIR SINGH AHUJA

TITLE: CLONING OF NOVEL GENE SEQUENCES EXPRESSED AND REPRESSED DURING WINTER DORMANCY IN THE APICAL BUDS OF TEA (CAMELLIA SINENSIS L. (O.) KUNTZE) BUSH

SPECIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Not applicable.

STATEMENTS REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

REFERENCE TO A MICROFICHE APPENDIX

[0003] Not applicable.

BACKGROUND OF THE INVENTION

1. Field of Invention

[0004] The present invention relates to cloning of novel gene sequences expressed and repressed during winter dormancy in the apical buds of *Camellia sinensis* L. (O.) Kuntze (hereinafter, referred to tea) bush. Particularly, this invention relates to identification, cloning and analysis of novel 3 prime (hereinafter called as 3') ends of the genes (gene in the present invention refers to the deoxyribonucleic acid (hereinafter known as, DNA) sequences that are expressed and repressed in winter-dormant apical buds of tea. 3' end refers to that end of DNA which has free hydroxyl group at 3rd position of the carbohydrate moiety of the DNA molecule.

2. Background and Prior Art References to the Invention

[0005] Tea (*Camellia sinensis* L. (O.) Kuntze) is a perennial plant grown in 31 countries located between 45°N-30°S and 150 °E-60°W. Being perennial, the plant experiences several cycles of summer, winter and rainy seasons. Apical bud and the associated two leaves (hereinafter, referred to BATS) are used for tea manufacture. BATS are harvested at frequent

interval, depending upon their availability. Prevailing environmental conditions (such as temperature, sun-shine hours and soil and atmospheric moisture etc.) and tea clone determine the availability of BATS for the purpose of harvesting.

[0006] A temperature of 30 °C and a day length of 13 hours are considered ideal for growth of the plant. As the temperature drops below 13 °C and day length becomes shorter than 11 hours and 15 minute, the growth and development of BATS is stopped. This phenomenon of suspension of the growth of BATS is termed as winter dormancy. Unlike deciduous plants, tea does not shed any leaf and remains green during dormancy period (Barua, D. N. 1989. Science and practice in tea culture. Tea Research Association, Calcutta, India. 509 p.). Winter dormancy is a universal phenomenon occurring in tea crops grown under the environmental conditions described above (Barua, D. N. 1989. Science and practice in tea culture. Tea Research Association, Calcutta, India. 509 p.). The following **Chart 1** illustrates the period of winter dormancy in various tea growing countries across the globe:

Chart 1

Country/ Place	**Dormancy Period**	**Location**
Georgia, U.S.S.R.	6 months	42 °N
Turkey	6 months	41 °N
Iran	5-6 months	37 °N
North–East India	3-4 months	26 °N
Mauritius	2-3 months	20 °S
Argentina	3-4 months	30 °S
Himachal Pradesh	5-6 months	37 °N

[0007] (Data adapted from Barua, D. N. 1989. Science and practice in tea culture. Tea Research Association, Calcutta, India. 509 p.; Dudeja, V. 1992. Tea Today 13: 1-5).

[0008] Temporary suspension of growth or the dormancy is not only limited to apical buds as in the case of tea but, is prevalent in other plats and systems as follows:

a) apical buds of *Taxus* species (Janes; Harry, W.; Gore; Gerald, E.; Wittman; Wayne, K.; Romen; Harry, T. July 1, 1997; US Patent # 5642587), beach (*Fagus sylvatica*; Heide, O.M. 1993. Physiol. Plant. 89: 187-191); grapevine (*Vitus vinifera*; Nir, G., Shulman, Y., Fanberstein, L. and Lavee, S. 1986. Plant Physiol. 81: 1140-1142; Or, E., Vilozny, I., Eyal, Y. and Ogrodovitch,

A. 2000. Plant Mol. Biol.43: 483-494); peach, (Marquat, C., Vandamme, M., gendraud, M. and Petel, G. 1999. Sci. Hort. 79: 151-162; Faye, F.and Floc'h, F. L. J. 1999. Plant Physiol. 154: 471-476); rose (*Rosa hybrida*; Bris, M. L., Michaux-Ferrière, N., Jacob, Y., Poupet, A., Barthe, P., Guigonis, J-M. and Page-Degivry, M-T. L. 1999. Aust. J. Plant Physiol. 26: 273-281); populus, (Rohde, A., Montagu, M. V. and Boerjan, W. 1996(*In* Somatic Cell Genetics and Molecular Genetics of Trees. eds Ahuja, M. R., Boerjan, W. and Neale, D.B. Kluwer Academic Publishers. Netherlands. pp 183-188); Jian, L-c., Li, P. H., Sun, L-h and Chen, T. H. H. 1997. J. Exp. Bot. 48: 1195-1207).

b) seeds of douglas fir (*Pseudotsuga menziesii*; Taylor, M. A., Davies, H. V., Smith, S. B. Abruzzese, A. and Gosling, P. G. 1993. J. Plant Physiol. 142: 120-123; Jarvis, S. B., Taylor, M.A., Bainco, J., Corbineau, F. and Davies, H. V. J. 1997. Plant Physiol. 151: 457-464); barley (*Hordeum vulgare*; Aalen, R. B. Opsahl-Ferstad, H-G., Linnestad, C. and Olsen, O-A. 1994. Plant J. 5: 385-396; Stacy, R. A. P., Munthe, E., Steinum, T., Sharma, B. and Aalen, R. B. 1996. Plant Mol. Biol. 31: 1205-1216); wild oat (*Avena fatua*; Li, B. and Foley, M., E. 1995. Plant Mol. Biol. 29: 823-831); tomato, (Groot, S. P. C., and Karssen, C. M. 1992. Plant Physiol. 99: 952-958); cheat (*Bromus secalinus*; Goldmark, P. J., Curry, J., Morris, C., F. and Walker-Simmons, M. K. 1992. Plant Mol. Biol. 19: 433-441); arabidopsis (*Arabidopsis thaliana*; Leon-Kloosterziel, K. M., Gil, M. A., Ruijs, G. J., Jacobsen, S. E., Olszewski, N. E., Schwartz, S. H., Zeevaart, J. A. D. and Koornneef, M. 1996. Plant J. 10: 655-661; Haslekas, C., Stacy, R. A. P., Nygaard, V., Culiáñez-Macià and Aalen 1998. Plant Mol. Biol. 36: 833-845); wheat (*Triticum aestivum*; Morris, C. F., Anderberg, R., J., Goldmark, P. J. and Walker-Simmons, M. K. 1991. Plant Mol. Biol. 1995: 814-821);

c) tubers of potato (*Solanum tuberosum*; Macdonald, M. M. and Osborne, D. J. 1988. Physiol. Plant. 73: 392-400)

d) corms of gradiolus (*Gladiolus grndiflorus*; Ginzberg, C. 1973. J. Exp. Bot. 24: 558-566), saffron crocus (*Crocus sativus* L.; Chrungoo, N. K. and Farooq, S. 1988. Acta Physiol. Plant. 10: 247-255)

e) Bulbs of onion (Abdel-Rahman, M. and Isenberg, F. M. R. 1974. J. agric. Sci. camb. 82: 113-116); tuberose (*Polyanthus tuberosa* L.; Nagar, P.K. 1995. Scientia Horticulturae. 63: 77-82); tulip (Reea, A R. 1981. Ann. Appl. Biol.

98: 544-548); yam (*Dioscorea spp*; Hasegawa, K. and Hashimoto, T. 1973. Plant Cell Physiol. 14: 369-377)

[0009] Thus, dormancy is exhibited by plant part(s) having meristematic tissue under unfavorable conditions, whether internal or external.

[0010] Modulation of dormancy has been and will continue to be a key issue in agriculture system. For example, if seeds or tubers or corms have reduced dormancy periods, before-time germination would lead to a loss to the growers if, the intention was to use them as sowing material. If intended for human or animal consumption, there will be a loss in the nutritional and or processing quality. In either of the cases, economic benefits will not be realized. On the contrary to this, an extended period of dormancy will lead to late germination or sprouting in the grower's field that would affect the crop performance and the crop yield.

[0011] Also, one of the major requirements of the tea industry is to have a tea plant with no or at least a reduced period of dormancy in the areas where this problem is prevalent. Nakayama, A. and Harada, S. (Bulletin of the Tea Research Station, Japan, 1962. 1: 28-39) and Barua, 1969 (Two and A Bud.16: 41-45) pointed out that low temperature coupled with short day length were responsible for inducing winter dormancy in tea. Hence, application of gibberellic acid, a plant growth regulator, was recommended to break dormancy. But the action of gibberellic acid was not persistent. Moreover, the results varied with the clones (Das, S. 1977. Ph.D. Thesis, Gauhati University, Assam; Rustogi, P. N. 1980. Two and A Bud. 21: 33). Studies in seeds, tubers and corms also resulted the similar conclusions (Aalen, R. B., Opsahl-Ferstad, H-G., Linnestad, C. and Olsen, O-A. 1994. Plant J. 5: 385-396; Bagni, N., Malucelli, B. and Torrigiana, P. 1980. Physiol. plant. 49: 341-345; DeBottini, G. A., Bottini, R. and Tizio, R. 1982. Oyton. 42: 115-121; Bewley, J.D. and Black, M. 1985. Physiology of development and germination. Plenum Press, New York. pp190-192; Delvallee, I., Paffen, A., and de Klerk, G.J. 1990. Physiol. Plant. 80: 431-436; Djilinov, D., Gerrits, M., Ivanova, A., Van Onikelen, H.A. and Dc Klerk, G.J. 1994. Physiol. Plant. 91: 639-644; Ginzberg, C. 1973. J. Exp. Bot. 24: 558-566; Kurashi, S., Daisuki, Y., Naoki, S. and Shigeki, H. 1998. J. Plant Growth Regul. 8: 3-10).

[0012] The above results dictated the adoption of molecular approach. A few dormancy related cDNAs have been cloned from seeds of *Bromus* and *Hordeum vulgare* using differential screening (Goldmark, P. J., Curry, J., Morris, C. F. and Walker-Simmons, M. K.

1992. Plant Mol. Biol. 19: 433-441; Aalen, R. B., Opsahl-Ferstad, H-G., Linnestad, C. and Olsen, O-A. 1994. Plant J. 5: 385-396.). Clone pBS128 was isolated from dormant *Bromus* seeds and the application of ABA to non-dormant seeds resulted in enhanced expression of this transcript (Goldmark, P. J., Curry, J., Morris, C. F. and Walker-Simmons, M. K. 1992. Plant Mol. Biol. 19: 433-441). This pBS128 from *Bromus secalinus*, which is maintained at a high level in dormant, but not in non-dormant hydrated seeds, has been suggested to play a role in the maintenance of embryo dormancy. Sequence analysis revealed that the encoded protein belongs to recently discovered group of antioxidants called peroxiredoxin (Li, B. and Forey, M.E. 1997. Trends in Plant Sci. 2: 384-389). The sequence analysis did not show any homology to the other reported genes. Interestingly, a transcript B15C present in the embryo of developing barley seeds showed 95% homology with the clone pBS128 (Aalen, R. B., Opsahl-Ferstad, H-G., Linnestad, C. and Olsen, O-A. 1994. Plant J. 5: 385-396.).

[0013] Differentially expressed partial cDNA fragments have been cloned from dormant and non-dormant wild oat embryos (Johnson, R. R., Cranston, H. J., Chaverra, M. E. and Dyer, W. E. 1995. Plant Mol. Biol. 28: 113-122). Out of several such clones, two clones namely AFD4 and AFN5 were found to be very interesting. The protein coded by AFD4 (protein Z analogue) was presumed to be a repressor of germination that maintains the seeds in dormant state. AFN5, which was expressed very early during imbibition, might code for a protein to be needed to signal or initiate early steps in germination (Johnson, R. R., Cranston, H. J., Chaverra. M. E. and Dyer, W. E. 1996. Plant dormancy, Physiology, Biochemistry and Molecular Biology. CAB International, Wallingford, UK. pp. 293-300). Other embryo specific genes that are induced by the application of ABA have also been identified (Hatzopoulos, P., Fong, F. and Sung, Z.R. 1990. Plant Physiol. 94: 690-695; Vance, V.B. and Huang, A.H.C. 1988. J. Biol. Chem. 263: 1476-1481), but their correlation with dormancy was not established.

[0014] The following **Chart 2** illustrates the status of information available on the phenomenon of dormancy in plants:

Chart 2:

Character, Property, Trait, Enzyme, Gene	**Criteria/ Objective**	**System adopted**	**Reference**
β-Amylase and starch	Starch catabolism affecting dormancy	Resting fronds (turions) of aquatic	Luka, Z. A., Xyländer, C.,

Character, Property, Trait, Enzyme, Gene	Criteria/ Objective	System adopted	Reference
phospphorylase		vascular plant greater duckweed (*Spirodela polyrhiza)*	Leeuwen, N. V., Schmidt, K-H and Appenroth, K-J. 1999. J. Plant Physiol. 154: 37-45
LEA genes	LEA gene expression affecting dormancy	Douglas fir seeds (*Pseudotsuga menziesii* (Mirb.) Franco)	Jarvis, S. B., Taylor, M. A., Bianco,` J., Corbineau, F. and Davies, H. V. J. 1997 Plant Physiol. 151: 457-464
Heat stable proteins	Heat stable proteins responsive to ABA	Embryonic axes of dormant wheat grains (*Triticum aestivum* L. cv Brevor)	Ried, J. L. and Walker-Simmons., M. K. 1990. Plant Physiol. 93: 662-667.
CDNA clones	Differentially expressed genes between dormant and non-dormant embryos	Embryos of *Avena fatua* L. caryopses	Johnson, R. R., Cranston, H. J., Chaverra, M. E. and Dyer, W. E. 1995. Plant Mol. Biol. 28: 113-122
Peroxiredoxin antioxidant gene	AtPer1, a peroxiredoxin antioxidant gene is related to dormancy	Seeds of *Arabidopsis thaliana* C24 ecotype, landsberg (wild type) and mutants, abi 3-1 and aba-1	Haslekas, C., Stacy, R. A. P., Nygaard, V., Culiáñez-Macià and Aalen 1998. Plant Mol. Biol. 36: 833-845
Antioxidant protein	PER1 peroxiredoxin, antioxidant is related to dormancy	Dormant seeds of Barley (*Hordeum vulgare* L.)	Stacy, R. A. P., Nordeng, T. W., Culiáñez-Macià, F. A. and Aalen, R. B. 1999. Plant J. 19: 1-8.
Catalase	Catalase activity during dormancy	One node cuttings of canes/or buds of *Vitis vinifera* L.	Nir, G., Shulman, Y., Fanberstein, L. and Lavee, S. 1986. Plant Physiol. 81: 1140-1142
Chilling and long days	Chilling and long days are required to break dormancy	Buds of *Fagus sylvatica*	Heide. O. M. 1993. Physiol. Plant. 89 : 187-191
α-amylase	Correlation of GA3 responsiveness and α-amylase secretion with dormancy.	Barley grains *(Hordeum distichum* L. cvs. Triumph and Kristina)	Schuurink, R. C., Sedee, N. J. A. and Wang, M. 1992. Plant physiol. 100: 1834-1839

Character, Property, Trait, Enzyme, Gene	Criteria/ Objective	System adopted	Reference
MRNA	Correlation of pBS128 transcript dormancy phenomenon	Embryo/or whole seeds of Bromus secalinas (Cheat) frozen at -20 °C to preserve dormancy and in some dormancy was dissipated by post harvest storage	Goldmark, P. J., Curry, J., Morris, C. F. and Walker-Simmons, M. K. 1992. Plant Mol. Biol. 19: 433-441
p34 kinase	Cell cycle status and $p34^{cdc2}$ Kinase expression during dormancy	Potato tubers stored at 3 °C, transferred to 20 °C after 120 days and 223 days of storage	Campbell, M.A., Suttle, J. C. and Sell, T. W. 1996. Physiol. Plant. 98: 743-752
GTP binding protein	GTP binding protein related to dormancy	Seeds of *Fagus sylvatica*	Nicolás, C., Nicolás, G. and Rodríguez, D.1998. Plant Mol. Biol. 36: 487-491
Cell cycle regulation during growth and dormancy	Accumulation of mRNAs corresponding to histones H2A and H4, ribosomal protein genes rpL27 and rpL34, MAP kinase, cdc2 kinase and cyclin B were analysed during growth-dormancy cycles	Peas (*Pisum sativum* L. cv. Alaska) were grown at 22 °C under 16h light/8h dark photoperiod	Devitt, M. and Stafstrom, J. P. 1995. Plant Mol. Biol. 29: 255-256
SNF like protein kinase GDBRPK	Up-regulation of GDBRPK a transcript for SNF-like protein involved in de-repression of meristemetic activity and release of dormanc	Buds of grapevines (*Vitis vinifera* cv. Perlette)	Or, E., Vilozny, I., Eyal, Y. and Ogrodovitch, A. 2000. Plant Mol. Biol. 43: 483-494
Synthesis of new proteins in the embryos of imbibed dormant seeds	Seeds of *Avena fatua* L. line AN265		Dyer, W. E. 1993. Physiol. Plant. 88: 201-211

Character, Property, Trait, Enzyme, Gene	Criteria/ Objective	System adopted	Reference
Inorganic pyrophosphatase	Related to non-dormancy	Hordeum cv. Triumph	Visser, K., Heimovaara-Dijkstra, Kijne, J. W. and Wang, M.1998. Plant. Mol. Biol. 37: 131-140
Peroxiredoxin antioxidant.	Related to dormancy and expressed during late development in aleurone and embryo of barley	Seeds of barley plants *(Hordeum vulgare* L.).	Stacy, R. A. P., Munthe, E., Steinum, T., Sharma, B. and Aalen, R. B.1996. Plant. Mol. Biol. 31: 1205-1216
MrnA and proteins	Changes mRNAs and proteins during dormancy and initial stages of germination	Seeds of Trollius ledebouri cv. Golden Queen	Bailey, P. C., Lycett, G. W. and Roberts, J. A. 1996. Plant Mol. Biol. 32: 559-564
Ca^{2++} and ultrastructure of buds	Altration in subcellular Ca^{2++} localization and ultrastructure of apical bud cells during development of dormancy	Poplar plants (*Populus deltoides* Bartr. Ex Marsh.)grown in greenhouse conditions	Jian, L-c., Li, P. H., Sun, L-h and Chen, T. H. H. 1997. J. Exp. Bot. 48: 1195-1207
Abscisic acid reponsive genes	Enhanced expression of five mRNAs in relation to dormancy	*Triticum aestivum* L. cv Brevor seeds	Morris, C. F., Anderberg, R. J., Goldmark, P. J. and Walker-Simmons, M. K. 1991. Plant Physiol. 95: 814-821
ABA	ABA synthesized in the bud arrests cell cycle in G2 phase	Two year old plants of *Rosa hybrida* L. cv. Ruidrilo Vivaldi	Bris, M. L., Michaux-Ferrière, N., Jacob, Y., Poupet, A., Barthe, P., Guigonis, J-M. and Page-Degivry, M-T. L. 1999. Aust. J. Plant Physiol. 26: 273-281

Character, Property, Trait, Enzyme, Gene	Criteria/ Objective	System adopted	Reference
ABA	ABA is required for dormancy maintenance	Wild type tomato (*Lycopersicon esculentum* Mill. Cv Money maker) and isoganic ABA deficient line *sit*^{w} isogenic GA-deficient line *gib-1* and isogenic recombinant line *gib-1 sit*^{w}	Groot, S. P. C. and Karssen, C. M. 1992. Plant Physiol. 99: 952-95
Ribosomal protein gene (pGB8)	Negatively related to dormancy	Seedling of pea (*Pisum sativum* L. cv Alaska)	Stafstrom, J. P. and Sussex, I. M. 1992. Plant Physiol. 100: 1494-1502
ABA	High level of ABA related to dormancy	Tuberose bulbs (*Polianthes tuberosa* L.)	Nagar, P. K. 1995. Sci.Hort. 63: 77-82.
Nucleic acids	Synthesis of nucleic acids and proteins during dormancy and early sprouting	Tubers of *Solanum tuberosum* L. cv. King Edward.	Macdonald, M. M. and Osborne, D. J. 1988 Physiol. Plant. 73: 392-400
B15C cDNA and its product.	B15C Expression in aleurone layer and embryo is related to dormancy	Anthers were cultured from the spikes of *Hordeum vulgare* cv. Dissa and perennial rye grass. Embryogenic and non-embryogenic material separated under microscope	Aalen, R. B., Opsahl-Ferstad, H-G., Linnestad, C. and Olsen, O-A. 1994. Plant J. 5: 385-396
Dehyrin and LEA genes	DF6 and DF77 cDNA clones related to dormancy	Seeds of Douglas fir (*Pseudotsuga menziesii*)	Jarvis, S. B., Taylor, M. A., Macleod, M. R., and Davies, H. V. 1996. J. Plant Physiol. 147: 559-566
Adenosine kinase	Enzyme activity affected by dormancy	Flower and vegetative buds peach tree (*Prunus persica* L.)	Faye, F. and and Floc'h, F. L. 1999. J. Plant Physiol. 154: 471-476
Proteins	Effect of stratification on gene expression during	Seeds of Doglas fir (*Pseudotsuga menziesii*)	Taylor, M. A., Davies, H. V., Smith, S. B.,

Character, Property, Trait, Enzyme, Gene	Criteria/ Objective	System adopted	Reference
	dormancy breakage		Abruzzese, A. and Gosling, P. G. 1993. J. Plant Physiol. 142: 120-123
Carbohydrate	Relation between carbohydrate absorption potentials and carbohydrate concentration	Vegetative buds of peach tree (*Prunus persica* L.)	Marquat, C., Vandamme, M., Gendraud, M. and Petél, G. 1999. Sci. Hort. 79: 151-162
Plant growth regulators	Application of exogenous plant growth regulators modulate dormancy	Onion bulbs cv. Elba	Abdel-Rahman, M. and Isenberg, F. M. R. 1974. J. agric. Sci. camb. 82: 113-116
Cell cycle specific promotor's activity	Chimeric β-glucuronidase (*gus*) genes under the control of cell cycle specific promotors	Buds of Populus (*Populus tremula x P. alba*)	Rohde, A., Montagu, M. V. and Boerjan, W. 1996. (*In* Somatic Cell Genetics and Molecular Genetics of Trees. eds Ahuja, M. R., Boerjan, W. and Neale, D.B. Kluwer Academic Publishers. Netherlands. pp 183-188)
Promotor's activity	Activity of *Agrobacterium rizogenes rolC* and CaMV *35S* promotor during annual cycle of growth and dormancy	Stem segments of hybrid aspen (*Populusbtremula* x *P. tremuloides*)	Nilson, O., Little, C. H. A., Sandberg, G. and Olsson, O. 1996. Plant Mol. Biol. 31: 887-895

[0015] There have been attempts to modulate dormancy process as follows:

(1) Bruce, D.; Eaton, C. D.; Schafer; Ronald, K. and Boise, I. D. in US Patent # 5811372 dated September 22, 1998 described a method to control the sprout formation in potatoes by prolonging the dormancy of potatoes during cold storage. Certain chemicals such as chloropham, carvone, bezothiazole were used with the average effective residue of CIPC (isopropyl 3--chlorophenyl-carbamate) on the tubers of approximately 16.6 ppm.

(2) Lulai; Edward, C. Orr; Paul, H., Glynn; Martin, T. in US Patent # 5436226 dated July 25, 1995 taught the suppression of sprouting of potato during storage in cool conditions by using jasmonates, a natural plant growth substance.

(3) Barry; Gerard Francis; Kishore; Ganesh Murthy; Stark; David Martin; Zalewaski; James Conrad in US Patent # 5648249 dated July 15, 1997 described a method of improving the quality of potatoes stored at reduced temperatures and a method of prolonging the dormancy of stored potato tubers. Transgenic potatoes were produced by transforming potato plants with a recombinant, double stranded DNA molecule comprising a promoter (cold-inducible promotor from potato or arabidopsis), a structural DNA sequence (which encoded a fusion polypeptide comprising an amino terminal plastid transit peptide and a *E.Coli* glgC ADPglucose pyrophosphorylase enzyme) and a 3' end nontranslated DNA sequence (functiond to cause translational termination and the addition of polyadenylated nucleotides to the 3' end of the RNA sequence). Promoter functioned to cause the production of an RNA sequence in tubers during cold storage and structural DNA sequence causes the production of an RNA Sequence. These transgenic potatoes tubers have been shown to exhibit prolonged dormancy period and inhibited sprouting at low temperature.

(4) Kawchuk, L. M.; Armstrong, J. D.; Lynch, D. R.; Knowles, N. R (1998) in Patent # WO9835051A1 dated Aug.13, 1998 disclosed to improve the quality of potatoes stored at low temperatures by suppressing the gene expression. Inventor produced transgenic potatoes exhibiting reduced levels of glucan L-type phosphorylase (GLTP) or glucan H-type phosphorylase (GHTP) enzyme activity with in the potato tubers stored at low temperatures. The transgenic potatoes had prolonged dormancy and increased storage life.

(5) Khan, A. A. (1994) in US Patent # 5294593 dated March 15, 1994 discussed the procedure for introducing dormancy in non-dormant seeds of vegetables, grasses, trees and shrubs and flowers, by treating them with tetcyclasis an inhibitor of giberellin biosynthesis.

(6) Janes, H. W., Gore, G. E., Wittman, W. K., and Romen, H. T in US Patent # 5642587 dated July 1, 1997 disclosed the procedure to enhance the production and recovery of taxol and taxotrene by increasing the production of biomass.

This was achieved by compressing the process of dormancy and by increasing the number of growing cycles within a greenhouse. To break dormancy, plants were placed in cold chamber under the conditions of no light and 85% of relative humidity for 30 days and then transferred to greenhouse (55 to 80 °F i.e., 13 and 27 °C; and relative humidity between 75% to 95%) for 40 day to grow. All the plants exposed to cold treatments showed more robust developed buds and overall plant growth. Untreated plants stopped growing and did not produce more growth after a period of time.

(7) Rieder, G. L. (US Patent # 4487625 dated December 11, 1984) discussed a method of interrupting bud dormancy in grapewine, a perennial crop, is described by spraying with 0.1 to10 weight percent aquous cyanamide solution. Homogenous development of blossoms and fruits with increasing crop yield were obtained.

(8) Illingworth, J. (December 2, 1997), in US Patent # 5693592 disclosed certain compositions of growth regulating compounds (selected from the group consisting of fatty acid esters, fatty acid amides, fatty alcohols, fatty alcohol alkoxides, phthalate esters, phthalic acid amides and imides and mixture of two or more thereof) for controlling dormancy break and blooming in dormant perennial plants such as stone fruits (plums, cheeies, peaches etc), pome fruits (apples and peers), vines, grapes, olives, temperate fruits (Kiwi fruit, figs morus), berries (strawberries, raspberries, cranberies, blackberries, loganberries) and nuts (almonds, walnuts, chestnuts) which require a necessary cold stimulus to break bud dormancy. Varied concentrations (0.5 to 50 % by weight) of different compositions of the above said growth regulating compounds were used in different plant species, in the dormant season with in 60 days before expected bud break. The invention was found to be useful to bring forward or setting back the time of bud break on fruit trees.

[0016] Below is specifically given a state of art knowledge with reference to the dormancy related genes from apical buds of the plants:

Reference may be made to document (1) by Or, E., Vilozny, I., Eyal, Y. and Ogrodovitch, A. 2000. Plant Mol. Biol. 43: 483-494, **wherein** is described the identification of a grape dormancy-breaking-related protein kinase (hereinafter referred to GDBRPK) transcript, which was up-regulated upon chemical induction of dormancy release by hydrogen

cyanamide. GDBRPK has a 976 base pair (hereinafter known as, bp) open reading frame that encodes for 325 amino acids. This is followed by a 241 bp 3'-untranslated region. GDBRPK, presumably, served as a sensor of stress signal.

Reference may be made to document (2) by Rohde, A., Montagu, M. V. and Boerjan, W. 1996. (*In* Somatic Cell Genetics and Molecular Genetics of Trees. eds Ahuja, M. R., Boerjan, W. and Neale, D.B. Kluwer Academic Publishers. Netherlands. pp 183-188), **wherein** it was concluded that the process of bud and seed dormancy may share similar events. It may be noted that the work did not include any differential gene expression pattern or protein profiling studies. The conclusion was based upon work on transgenic *Populus* harboring chimeric β-glucuronidase gene under the control of cell cycle specific promoters. The studies, therefore, did not reveal any mechanism of bud dormancy *per se* or yielded any gene(s) related to the bud dormancy.

The drawbacks in the prior art are:

(a) Earlier efforts to modulate the dormancy by exposing the plants to low temperature condition is not possible for the plants standing in the field. Particularly, for the plants of, for example, *Taxus*, plums, peaches, apples, figs, morus, almonds, walnuts and tea etc., which being tree can not be grown in pots

(b) Earlier efforts to modulate the dormancy by spraying chemical formulations will not be environmental friendly and hence would contribute to environmental pollution

(c) There are no gene(s) till today, which have been cloned from dormant or the non-dormant buds under natural conditions. There is only one report on the isolation of the gene cloned from the buds of grapevine (*Vitis vinifera* cv. Perlette). However, the cloned gene is the one that is induced in response to the application of hydrogen cyanamide (Or, E., Vilozny, I., Eyal, Y. and Ogrodovitch, A. 2000. Plant Mol. Biol. 43: 483-494)

(d) In some of the cases, the gene of a particular enzyme has been taken from microorganism to be placed in the transgenic plant. This causes environmental concern and the hazard. The desirable feature would be to take the relevant gene from the plant system growing in similar ecosystem *per se* for the purpose of modulating the dormancy phenomenon

(e) There is no spectrum of the gene(s) expressed and repressed during the dormancy process

(f) The important aspect which has been not been considered in earlier invention is the same genetic make up of control and the treated tree species for the purpose of identification and cloning of the differentially expressed gene(s).

[0017] The above drawbacks have been eliminated for the first time in a simple and reliable manner by the present invention, which is not so obvious to the person skilled in the art.

BENEFITS OF THE INVENTION

[0018] A benefit of the present invention is the cloning of novel DNA sequences expressed and repressed during winter dormancy in the apical buds of tea (*Camellia sinensis* L. (O.) Kuntze) bush growing under field conditions.

[0019] Yet another benefit of the present invention is to ensure the same genetic make up of the plant considered for the purpose of identification and cloning of novel DNA sequences expressed and repressed during winter dormancy in the apical buds of tea (*Camellia sinensis* L. (O.) Kuntze) bush growing under field conditions.

[0020] Yet another benefit of the present invention is to generate a spectrum of the gene(s) expressed and repressed during the process of winter dormancy in the apical buds of tea (*Camellia sinensis* L. (O.) Kuntze) bush growing under field conditions.

[0021] Yet another benefit of the present invention is the identification of 3' ends of the gene(s) expressed and repressed during the process of winter dormancy in the apical buds of tea bush growing under field conditions.

[0022] Yet another benefit of the present invention is the confirmation of the identified 3' ends of the differentially expressed gene(s) for establishing differential expression during winter dormancy in the apical buds of tea bush growing under field conditions.

[0023] Still another benefit of the present invention is the cloning of the identified 3' ends of the differentially expressed gene(s).

[0024] Yet another benefit of the present invention is the sequencing of the identified 3' ends of the cloned gene.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

Figure 1 represents Total RNA isolated from the non-dormant (ND) and dormant (D) buds of tea. M represents RNA marker.

Figure 2 represents cDNA synthesized from the total RNA isolated from the ND and D buds of tea. $T_{11}A$, $T_{11}C$ and $T_{11}G$ represent the primers used for the purpose of cDNA synthesis using total RNA in three separate reactions. M lane contains DNA molecular weight marker.

Figure 3 represents spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 4 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 5 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 6 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 7 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 8 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 9 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of

each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 10 represents further spectrum of 3' ends of the expressed and repressed genes in ND and D apical buds of tea using the primer combinations as defined at the bottom of each lane. Number on the top of each lane represents lane number. Arrow indicates differential expression.

Figure 11 represents amplification of the differentially expressed 3' ends of the gene after eluting from the sequencing (denaturing polyacrylamide) gels as shown in figures 3-10. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 12 represents further amplification of the differentially expressed 3' ends of the gene after eluting from the sequencing (denaturing polyacrylamide) gels as shown in figures 3-10. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 13 represents further amplification of the differentially expressed 3' ends of the gene after eluting from the sequencing (denaturing polyacrylamide) gels as shown in figures 3-10. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 14 represents further amplification of the differentially expressed 3' ends of the gene after eluting from the sequencing (denaturing polyacrylamide) gels as shown in figures 3-10. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 15 represents amplification after cloning of the eluted differentially expressed 3' ends of the gene as mentioned in Figures 11-14. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the

dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 16 represents amplification after cloning of the eluted differentially expressed 3' ends of the gene as mentioned in Figures 11-14. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 17 represents amplification after cloning of the eluted differentially expressed 3' ends of the gene as mentioned in Figures 11-14. The first number at the top of each lane represents the lane number as mentioned in Figures 3-10. The second number followed by the dot represents the number of differentially expressed band as counted from the top of the respective lane as mentioned in figures 3-10. M represents DNA size marker.

Figure 18 represents confirmation of differential expression of the cloned 3' ends of the gene through northern hybridization.

Figure 19 represents further confirmation of differential expression of the cloned 3' ends of the gene through northern hybridization using 2 more clones.

Figure 20 represents expression of the identified, cloned 3' ends of gene number 31.2 from ND apical buds in ND, D and forced ND apical buds (gibberellic acid, GA_3, was applied onto the D buds during winter season to force the buds to enter into non-dormancy stage).

SUMMARY OF THE INVENTION

[0025] Accordingly, the present invention provides:

(a) novel DNA sequences expressed and repressed during winter dormancy in the apical buds of *Camellia sinensis* L. (O.) Kuntze (tea) bush under field conditions,

(b) cloning of such sequences have been from the same genetic make up of the tea bush,

(c) a spectrum of 3' ends of the expressed and repressed genes in non-dormant and dormant apical buds of tea bush growing under field conditions,

(d) confirmation of the identified 3' ends of the differentially expressed gene(s) for establishing differential expression during winter dormancy in the apical buds of tea bush growing under field conditions,

(e) method to correlate the identified gene with the dormancy of tea buds under field conditions, and

(f) sequencing of the cloned 3' ends of the differentially expressed gene(s) showed uniqueness in terms of novel sequences not deposited in the data bank so far.

DETAILED DESCRIPTION OF THE INVENTIONS

[0026] Accordingly, the present invention provides novel DNA sequences expressed and repressed during winter dormancy in the apical buds of *Camellia sinensis* L. (O.) Kuntze (tea) bush or a tree species, said sequences comprising sequence ID 1 to 4 as shown here below:

SEQ ID NO: 1

5'- ATCGCCGTAA TTGCCATGTT TTCCCTCTCA CCGGAATCCT ACG TTATCC CCTTACCTTC GTGAACATTA CAGTAGGAAT CGGTGGTCCA ATTATCAACT TAATTTTGGG CGCATCTGTT CGTGTTAACT AGAAGCCATG TATACATACA ATACAACATG GTTCACTCCT CCTACAGATT ATGAGTTGAA CTTTTATAAT AAGTTGTAAT AATGGCTTCT GAATAAGGAG AAGAGGAGCC TCTGTTTGTT TTACTTATTA CAGATGTGAT ATCGTTCAAC AACTTTGATT CTGCGAAAAA AAAAA-3'

SEQ ID NO: 2

5'- AGAAGTACCT GAAAGGAAGC TTAACGAGGT GAACATCCAT TGCAGCCAGC CCTGGAATCT GTACAGGGCA ACTCTGAACC GGAATTATTT TAATAACCCG TGGGCAATGA TTGCAATTAT GGCTCGTTTG GTATTACTTC TACTCACTTA GACACAACTG TATTTACGGT TTTCGCTGGA ATTGTAATTG TTGGAGCGAC AAAATAGATG GTCACAACTT ATTGGTGAGA GTATCAGTGT GCTCTTCTTT ATCGTCTTTA ACTCTCCGTG GTAATTACTT TGACAATATT CATACAT -3'

SEQ ID NO: 3

5'- GAGACTCAGC TCAGCAATCA TGTTCTAAGT GAATGTCACT CTATCGCCTT CTTGTCCCTC TTAGACATAC TACATCCTCA TTCTGCTAGA AATGAACTCA TGTAGGTTTT GAAGTTGGGA ACTTTTGAAA CTGTGTTGTT TGGGTGCTGT CTGTTATACA ATTCTCTCAA CTGCGGAGAA TTGACGTTGG TTGTAGTGGA ATTCAACACT TGGGTTTTGT TCTTAGTTAA AAAAAAAAA-3'

SEQ ID NO: 4

5'-ATAGCTTAGT CACGTGTCTC TTGAGAATGG ACTACGTAGT TGTTAAGTTG GGTGATCAGA AGGCGTTGAT GATGAATGTA TGAAGCAGAG ACTACTGAAT GTAATTTTGT TGTTGAAAGA TGAATGATTT ATTAATGCCT GCATATCTTT CTATTGTTTG ATGCCAAACC TTTGGGCACA TTTTTTCTTT CTTTTTGTGA TAATGTTCTC TTCTTGCAAA AAAAAAAA-3'

[0027] In an embodiment of the invention, the novel DNA sequences, which are cloned from the tea bush of the same, genetic make up.

[0028] In another embodiment of the invention, the novel DNA sequences, which are cloned from the tea bush of the same genetic make up growing under field conditions.

[0029] In another Novel DNA sequences as claimed in claim 1, are associated with winter dormancy in tea.

[0030] In still another embodiment of the invention, the novel DNA sequences, which are cloned by any methods but not limited to, subtractive hybridization and differential screening.

[0031] In yet another embodiment of the invention, the nucleotide sequence of the DNA is given in SEQ ID NO 1.

[0032] In yet another embodiment of the invention, the nucleotide sequence of the DNA as is given in SEQ ID NO 1 is overexpressed only in non-dormant apical buds of tea.

[0033] In yet another embodiment of the invention, the nucleotide sequence of the DNA is given in SEQ ID NO 2

[0034] In yet another embodiment of the invention, the nucleotide sequence of the DNA as is given in SEQ ID NO 2 is expressed only in non-dormant apical buds of tea.

[0035] In yet another embodiment of the invention, the nucleotide sequence of the DNA is given in SEQ ID NO: 3

[0036] In yet another embodiment of the invention, the nucleotide sequence of the DNA as is given in SEQ ID NO 3 is expressed only in non-dormant apical buds of tea.

[0037] In yet another embodiment of the invention, the nucleotide sequence of the DNA is given in SEQ ID NO: 4

[0038] In yet another embodiment of the invention, the nucleotide sequence of the DNA as is given in SEQ ID NO 4 is expressed only in dormant apical buds of tea.

[0039] In yet another embodiment of the invention, the novel sequences are capable of being cloned to full-length cDNA.

[0040] In yet another embodiment of the invention, the novel sequences are capable of being cloned to full length genomic DNA.

[0041] In yet another embodiment of the invention, the novel sequences are capable of being cloned to important sequences, such as but not limited to, promoter sequences and regulatory sequences etc.,

[0042] Tea bushes, belonging to chinary type, growing and maintained in the tea farm of the Institute of Himalayan Bioresource Technology, Palampur (32° 04' N, 76 ° 29' E; altitude, 1300 m) were selected. Tea plants belonging to other types namely, assamica and combod could have also been selected. However, due to predominance of the chinary type in Kangra region of the Himachal Pradesh, this particular clone was selected.

[0043] In a preferred embodiment of the present invention the genetic make of the plant, considered for the purpose of identification and cloning of novel DNA sequences expressed and repressed during winter dormancy in the apical buds of tea (*Camellia sinensis* L. (O.) Kuntze) bush growing under field conditions was ensured to be the same. This is important for a plant like tea. Tea is a highly cross-pollinated plant and grown by the seed. This leads to enormous plant to plant heterogeneity due to differential genetic make up. Each seed raised plant of tea is regarded a clone in itself. Particularly, for the inventions related to compare gene expression pattern for the purpose of cloning the differentially expressed gene in response to an altered environmental condition, such as is the purpose of the present invention of comparing and cloning the expressed and repressed gene(s) from the control and a winter dormant plant, it is absolutely essential that the plants experiencing altered environmental conditions have the same genetic make up. Having realized this important aspect, only one bush of tea belonging to chinary type was selected.

[0044] In another embodiment of the present invention the apical buds of tea measuring 1.2-1.5 cm (in length); 0.5-0.6 cm (perimeter) and were collected during non-dormant season (in the month of April) at 10.00 O'clock in the morning from only one bush. Apical buds during non-dormant season grow to open into the leaves. These are referred to as non-dormant (hereinafter known as ND) buds and are considered "control" within the scope of the

present invention. The bush yielded around 100 numbers of apical buds. Apical buds were washed with diethyl pyrocarbonate (hereinafter known as DEPC) treated water [to prepare DEPC treated water, DEPC was added in distilled water to a final concentration of 0.1% followed by autoclaving (i.e. heating at 121 °C under a pressure of 1.1 kg per square centimeters) after an overnight incubation.], harvested and immediately dipped in liquid nitrogen to freeze the cellular constituents for ceasing the cellular activities.

[0045] Tea bush became dormant from the month of November onwards. The apical buds during this period do not grow in the size to open into the leaves and are referred to as dormant (hereinafter known as D) buds. The same bush, from which the ND buds were collected, was used to collect the D buds. D buds, measuring the similar dimensions as for the ND buds, were plucked and stored essentially as described for ND buds.

[0046] Collection of ND and D buds from the same bush ensured the same genetic make up of the tissue under consideration.

[0047] In another embodiment of the present invention total RNA from ND and D buds was isolated and the "differential display technique" (Liang, P., Zhu, W., Zhang, X., Guo, Z., O'Connell, R., Averboukh, L., Wang, F. and Pardee, A. B. 1994. Nucleic acids Res. 22: 1385-1386) was employed to generate a spectrum of 3' ends of the expressed and repressed genes in ND and D buds of tea

[0048] In an advantageous embodiment of the present invention 3' ends of the expressed and repressed genes in ND and D buds of tea were ligated into a vector to yield a recombinant plasmid, which upon transformation into a suitable *E. coli* host resulted into a clone. Vector, in the present invention refers to the sequence of DNA capable of accepting foreign DNA and take the form of a circular plasmid DNA that shows resistance to a given antibiotic.

[0049] In yet another embodiment of the present invention the gene cloned was tested for its expression or repression in ND and D buds of tea to define association of the cloned gene with the dormancy process.

[0050] In another embodiment of the present invention the gene was sequenced using the dideoxy chain termination method (Sanger, F. S., Nicklen, S. and Coulson, A. R. 1977. Proc. Natl. Acad. Sci. USA. 74: 5463-5467) to figure out the uniqueness of the gene.

[0051] One more embodiment of the invention, wherein the sequence data is used for obtaining, important information on the gene regulation.

[0052] In another embodiment of the invention, the novel genes are used to modulate winter dormancy in plants after transferring these genes using the techniques such as, but not limited to, *Agrobacterium* mediated transformation and Biallistic medited transformation

[0053] In another embodiment of the invention, it is possible to modulate winter dormancy using the novel genes in the plants such as, but not limited to, tea, plums, cherries, peaches, *Taxus*, apples, peers, vines, grapes, olives, Kiwi fruit, figs, morus, strawberries, raspberries, cranberies, blackberries, loganberries, almonds, walnuts and chestnuts after transferring these genes using the techniques such as , but not limited to, *Agrobacterium* mediated transformation and bialistic medited transformation.

[0054] In still another embodiment of the invention, use of sequence data of the novel genes for obtaining important information on the gene regulation to be exploited to regulate gene expression in transgene.

[0055] In still another embodiment of the invention relates to use of cDNAs and the genomic DNAs for synthesizing unique proteins and in addition use of unique proteins for raising antibodies.

[0056] In yet another embodiment of the invention relates to use of the present antibodies as probe to look for the similar proteins in other plants, animal and/or microbial systems or the like.

[0057] In yet another embodiment of the invention relates to use of novel sequences, cDNAs and the genomic DNAs of the present invention as probe to look for the sequences of nucleotides in other plants, animal and/or microbial systems and the like.

[0058] In yet another embodiment of the invention relates to use of novel sequences, cDNAs and the genomic DNAs of the present invention, as probe to look for the expression of these sequences of nucleotides in other plants, animal and/or microbial systems and the like.

[0059] In yet another embodiment of the invention relates a method to correlate the identified gene with the process of dormancy of tea buds as described for sequence ID 1 is unique.

[0060] In yet another embodiment of the invention relates a method which, can be applied to other sequence ID as well.

[0061] In yet another embodiment of the invention relates to a method which, can be applied to other crops such as, but not limited to plums, cherries, peaches, *Taxus*, apples, peers, vines, grapes, olives, Kiwi fruit, figs, morus, strawberries, raspberries, cranberries, blackberries, loganberries, almonds, walnuts and chestnuts as well for correlating similar genes.

[0062] The present invention will be illustrated in greater details by the following examples. These examples are presented for illustrative purposes only and should not be construed as limiting the invention, which is properly delineated in the claims.

Example 1

RNA Isolation, digestion of RNA with DNase 1, quantification of RNA and gel-electrophoresis:

To ensure a high quality of ribonucleic acid (hereinafter known as, RNA) from ND and D buds of tea bush, RNeasy plant mini kits (purchased from M/s. Qiagen, Germany) were used. Manufacturer's instructions were followed to isolate RNA. RNA was quantified by measuring absorbance at 260 nm and the purity was monitored by calculating the ratio of absorbance meaasured at 260 and 280 nm. A value >1.8 at 260/280 nm was considered ideal for the purpose of present investigation. The formula used to calculate RNA concentration and yield was as follows:

Concentration of RNA (μg/ml) $=A_{260}$ (absorbance at 260 nm) x 40 x dilution factor

Total yield (μg) = concentration x volume of stock RNA sample

To check the intigrity of RNA, 5-6 ℓg of RNA in 4.5 μl was diluted with 15.5 μl of M1 solution (2 μl of 5X MOPS buffer, 3.5 μl of formaldehyde, and 10 μl of formamide [5X MOPS buffer: 300 mM sodium acetate, 10 mM MOPS (3-{N-morpholino]propanesulfonic acid}, 0.5 mM ethylene diamine tetra-acetic acid (EDTA)]and incubated for 15 minutes at 65 °C. RNA was loaded onto 1.5% formaldehyde agarose-gel after adding 2 μl of formaldehyde-

gel loading buffer [50% glycerol, 1mM EDTA (pH, 8.0), 0.25% bromophenol blue, 0.25% xylene cyanol FF], and electrophoresed at 72 volts in 1X MOPS buffer (60 mM sodium acetate, 2 mM MOPS, 0.1 mM EDTA), (Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, NY).

To remove the residual DNA, RNA (10-50 ℓg) was digested using 10 units of DNase I, in 1X reaction buffer [10X reaction buffer: 100 mM Tris-Cl (pH, 8.4), 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin] at 37 °C for 30 minutes (Message clean kit from M/s. GenHunter Corporation, USA). DNase I was precipitated by adding PCI (phenol, chloroform, isoamylalcohol in ratio of 25:24:1) and RNA present in the aqueous phase was precipitated by adding 3 volumes of ethanol in the presence of 0.3 M sodium acetate. After incubating for 3 hours at –70 °C, RNA was pelleted, rinsed with chilled 70 % ethanol and finally dissolved in 10 µl of RNase free water. DNA-free-RNA thus obtained was quantified and the integrity was checked as above. The quality of RNA is depicted in Figure 1. Although we have used Rneasy colmns from M/S Quiagen, Germany, the other procedure can also be used to isolate from the apical buds of tea.

Example 2

Conversion of mRNA into complementary DNAs (hereinafter referred to cDNAs) by Reverse Transcription (hereinafter referred to RT):

0.2 µg of DNA-free-RNA from dormant and non-dormant samples was reverse transcribed in separate reactions to yield cDNAs using an enzyme known as reverse transcriptase. The reaction was carried out using 0.2 µM of $T_{11}M$ primers (M in $T_{11}M$ could be either $T_{11}A$, $T_{11}C$ or $T_{11}G$), 20 µM of dNTPs, RNA and RT buffer [25 mM Tris-Cl (pH, 8.3), 37.6 mM KCl, 1.5 mM $MgCl_2$ and 5mM DTT]. In the present invention dNTP refers to deoxyadenosine triphosphate (hereinafter reffered to dATP), deoxyguanosine triphosphate (hereinafter reffered to dGTP), deoxycytidine triphosphate (herein after reffered to dCTP) and deoxythymidine triphosphate (hereinafter reffered to dTTP). Three RT reactions were set per RNA sample for the corresponding $T_{11}M$ primer. The reactions were carried out in a thermocycler (model 480 from M/s Perkin-Elmer). Thermocycler parameters chosen for transcription were 65 °C for 5 minutes, → 37 °C for 60 minutes, → 75 °C for 5 minutes, → 4 °C. 100 units of reverse transcriptase was added to each reaction after 10 minute incubation at 37 °C and reaction then continued for rest of the 50 minutes. This yielded cDNAs as is shown

in figure 2. Two different RNA (ND and D) in combination with 3 $T_{11}M$ primers yielded a total of 6 reactions depicting 6 different classes of cDNAs. The use of 3 different $T_{11}M$ primers divided the whole RNA population into 3 sub-classes depending upon the anchored base M, which was either A, C or G (Reverse transcription system was a component of RNAimage kit from M/s. GenHunter Corporation, USA).

Example 3

Generation of a spectrum of differentially expressed genes through differential display technique: Identification of differentially expressed gene(s):

Different sub-classes of cDNA from dormant and non-dormant RT product as obtained in Example 2 were amplified in the presence of a radiolabelled dNTP to label the amplified product through polymerase chain reaction (hereinafter known as PCR; PCR process is covered by patents owned by Hoffman-La Roche Inc.). Radioactive PCR was carried out in 20 µl reaction mix containing a (1) reaction buffer [10 mM Tris-Cl (pH, 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin], (2) 2 µM dNTPs, (3) 0.2 µM $T_{11}M$ and (4) 0.2 µM arbitrary primers (chemicals 1 to 4 were purchased from M/s. GenHunter Corporation, Nashville, USA as a part of RNAimage kit), 0.2 µl $\alpha[^{33}P]$ dATP (~2000 Ci/mmole, purchased from JONAKI Center, CCMB campus Hyderabad, India), and 1.0 units of *Thermus aqueticus* (hereinafter referred to Taq) DNA Polymerase (purchased from M/S. Qiagen, Germany). 30 µl of autoclaved mineral oil was overlaid at the top of each reaction to avoid alteration in volume due to evaporation. $T_{11}M$ primer in each reaction was the same that was used to synthesize cDNA. Parameters choosen were: 40 cycles of 94 °C for 30 seconds,→ 40 °C for 2 minutes,→ 72 °C for 30 seconds; and 1 cycle of 72 °C for 5 minutes and final incubation at 4 °C.

Amplified products were fractionated onto a 6% denaturating polyacrlamide gel. For the purpose 3.5 µl of each of amplified product was mixed with 2 µl of loading dye [95% formamide, 10 mM EDTA (pH, 8.0), 0.09% xylene cyanol FF and 0.09% bromophenol blue], incubated at 80 °C for 2 minutes and loaded onto a 6% denaturating polyacrlamide gel [denaturating polyacrlamide gel: 15 ml of acrylamide (40 % stock of acrylamide and bisacrylamide in the ratio of 20:1), 10 ml of 10X TBE, 40 ml of distilled water and 50 g urea]. Electrophoresis was performed using 1X TBE buffer [10 X TBE: 108g Tris base, 55g boric acid and 40 ml of 0.5 M EDTA (pH, 8.0)] as a running buffer at 60 watts until the xylene cyanol (the slower moving dye) reached the lower end of the glass plates. Size of the

larger plate of the sequencing (denaturing polyacylamide) gel apparatus was 13 X 16 inch. After the electrophoresis, one of the glass plates was removed and the gel was transferred onto a 3 MM Whattman filter paper. Gel was dried at 80 °C overnight and exposed to Kodak X-ray film for 2-3 days. Before exposing to X-ray film, corners of the dried gel were marked with radioactive ink for further alignment. Figures 3 to 11 show the spectrum of differentially expressed genes in ND and D apical buds of tea as was seen after developing the film. After developing the gel, film was analyzed for differential expressed bands between ND and D signals.

Sequences of the primers used for differential display were as follows (purchased from M/s. GenHunter Corporation, USA as a prt of RNA image kit) :

$T_{11}M$ (anchored) primers	**Primer sequence**
$T_{11}A$	5'-AAGCTTTTTTTTTTTTA-3'
$T_{11}C$	5'-AAGCTTTTTTTTTTTTC-3'
$T_{11}G$	5'-AAGCTTTTTTTTTTTTG-3'
Arbitrary Primers	**Primer Sequence**
AP1	5'-AAGCTTGATTGCC-3'
AP2	5'-AAGCTTCGACTGT-3'
AP3	5'-AAGCTTTGGTCAG-3'
AP4	5'-AAGCTTCTCAACG-3'
AP5	5'-AAGCTTAGTAGGC-3'
AP6	5'-AAGCTTGCACCAT-3'
AP7	5'-AAGCTTAACGAGG-3'
AP8	5'-AAGCTTTTACCGC-3'
AP33	5'-AAGCTTGCTGCTC-3'
AP34	5'-AAGCTTCAGCAGC-3'
AP35	5'-AAGCTTCAGGGCA-3'
AP36	5'-AAGCTTCGACGCT-3'
AP37	5'-AAGCTTGGGCCTA-3'

AP38	5'-AAGCTTCCAGTGC-3'
AP39	5'-AAGCTTTCCCAGC-3'
AP40	5'-AAGCTTGTCAGCC-3'
AP65	5'-AAGCTT CAAGACC-3'
AP66	5'-AAGCTT GCCTTTA-3'
AP67	5'-AAGCTT TATTTAT-3'
AP68	5'-AAGCTT CTTTGGT-3'
AP69	5'-AAGCTT AATAACG-3'
AP70	5'-AAGCTT TCATATG-3'
AP71	5'-AAGCTT GTAGTAA-3'
AP72	5'-AAGCTT TCAAAGA-3'

Example 4

Reamplification of cDNA Probes:

Cloning the differentially expressed bands required elution of the same from the denaturating polyacrylamide gel and further amplification to yield substantial quantity of DNA for the purpose of cloning. Autoradiogram (developed X-ray film) was oriented with the dried gel aided with radioactive ink and the identified differentially expressed band (along with the gel and the filter paper) was cut with the help of a sterile sharp razor. DNA was eluted by incubating in 100 µl of sterile dH_2O for 10 min in an eppendorf tube, followed by boiling for 10 minutes. Paper and gel debris were pelleted by spinning at 10,000 rpm for 2 min and the supernatant containing DNA was transferred into a new tube. DNA was precipitated with 10µl of 3M sodium acetate, pH,5.5, 5 µl of glycogen (stock; 10 mg/ml) and 450 µl of ethanol. After incubation at –70 °C for overnight, centrifugation was performed at 10, 000 rpm for 10 min at 4 °C and pelleted DNA was rinsed with 85% ethanol. DNA pellet was dissolved in 10 µl of sterile distilled water.

Eluted DNA was amplified using the same set of $T_{11}M$ and arbitrary primer that was used for the purpose of performing differential display as in the Example 3. Also, the PCR conditions were the same except that dNTP concentration was 20 µM instead of 2 µM and no

isotopes was added. Reaction was up-scaled to 40 µl and after completion of PCR, 30 µl of PCR sample was run on 1.5% agarose gel in TAE buffer (TAE buffer: 0.04 M Tris-acetate, 0.002 M EDTA, pH 8.5) containing ethidium bromide (final concentration of 0.5 µg/ml). Rest of the amplified product was stored at –20 °C for cloning purposes (see Figures 11-14).

Example 5

Cloning of re-amplified PCR products:

Re-amplified PCR products as obtained in example 4 were ligated in 300 ng of insert-ready vector called as PCR-TRAP® vector using 200 units of T4 DNA-ligase in 1X ligation buffer (10X ligase buffer: 500 mM Tris-Cl, pH 7.8, 100 mM MgCl2, 100 mM DTT, 10 mM ATP, 500 µg/ml BSA). Vector and the other chemicals required were purchased from M/s. GenHunter Corporation, Nashville, USA as PCR-TRAP® cloning system. Ligation was performed at 16 °C for 16 hours in a thermocycler model 480 from M/s. Perkin Elmer, USA. Ligation of the PCR product into a vector such as above yields to a circularized plasmid. The process of ligation of the foreign DNA, such as the PCR product in the present invention, into a suitable vector, such as PCR-TRAP® vector in the present invention, is known as cloning. There is a range of other vectors that are commercially available or otherwise that suits the cloning work of PCR products and hence may be used. The plasmid, as per the definition, is a closed circular DNA molecules that exists in a suitable host cell such as in Escsherichia coli (hereinafter referred to E. coli) independent of chromosomal DNA and may confer resistance against an antibiotic. PCR-TRAP® vector resulting plasmid confers resistance against tetracycline.

Ligated product or the plasmid needs to be placed in a suitable E. coli host for its multiplication and propagation through a process called transformation. Ligated product (10 µl) as obtained above was used to transform 100 µl of competent E. coli cells (purchased from M/s. GenHunter Corporation USA as a part of PCR-TRAP cloning system). Competent means the E. coli cells capable of accepting a plasmid DNA. For the purpose, ligated product and competent cell were mixed, kept on ice for 45 minutes, heat shocked for 2 minutes and cultured in 0.4 ml of LB medium (LB: for 1 litre: 10g tryptone, 5g yeast extract, 10g sodium chloride) for 4 hours. 200 µl of transformed cells were plated onto LB-tetracyclin (for 1 litre: 10g tryptone, 5g yeast extract, 10g sodium chloride, and tetracyclin added to a final concentration of 20 µg/ml) plates and grown overnight at 37 °C. Colonies were marked and single isolated colonies were restreaked on to LB-tetracyclin plates to get colonies of the

same kind. Conferral of tetracyclin resistance to E. coli cells apparently suggests that the PCR product i.e. the identified gene has been cloned.

In whole of the above process, the selection of T11M primer will amplify the poly A tail region of mRNA. Poly A tail is always attached to 3' end of the gene and hence T11M primer in combination with an arbitrary primer would always yield 3' region of the gene.

Example 6

Checking the size of the PCR product :

Once the gene has been cloned and the *E. coli* has been transformed, it becomes imperative to check if the plasmid has received right size of the PCR product. This can be accomplished by performing colony PCR wherein the colony is lysed and the lysate is subsequently used to perform PCR using the appropriate primers. Amplified product is then analysed on an agarose gel.

Single isolated colonies were picked up from re-streaked plates (Example 5) and lysed in 50 µl colony lysis buffer (colony lysis buffer: TE (Tris-Cl 10 mM, 1 mM EDTA, pH 8.0) with 0.1% tween 20) by boiling for 10 minutes. Cell debris were pelleted and the supernatant or the colony lysate containing the template DNA was used for PCR. PCR components were essentially the same as in example 4 except that in place of $T_{11}M$ and arbitrary primers Lgh (5'-CGACAACACCGATAATC-3') and Rgh (5'-GACGCGAACGAAGCAAC-3') primers (specific to the vector sequences flanking the cloning site) were used and 2 µl of the colony lysate was used in place of eluted DNA. Also the reaction volume was reduced to 20 µl. PCR conditions used for colony PCR were, 94 °C for 30 seconds, → 52 °C for 40 seconds, → 72 °C for 1 minute for 30 cycles followed by 1 cycle of 5 min extension at 72 °C and final soaking into 4 °C. Amplified product are run on 1.5% agarose gel along with molecular weight marker and analyzed for correct size of insert. While using Lgh and Rgh flanking primers, the size of the cloned PCR product was larger by 120 bp due to the flanking vector sequence being amplified (See Figures 15-17).

Example 7

Confirmation of the differential expression by northern blotting

PCR products cloned above represent 3' end of the differentially expressed genes. Within the scope of the present invention, these cloned fragments of DNA will be called as genes. Since differential display invariably leads to false positives i.e. apparently

differentially expressed genes (Wan, J. S. and Erlander, M. G. 1997. Cloning differentially expressed genes by using differential display and subtractive hybridization. *In* Methods in Molecular Biology. Vol. 85: Differential display methods and protocols. Eds. Liang, P. and Pardee, A. B. Humana press Inc., Totowa, NJ, pp 45-68), a confirmatory test through northern analysis is mandatory to ascertain differential expression between ND and D apical buds. Northern analysis requires preparation of a radio-labelled probe followed by its hybridization with denatured RNA blotted onto a membrane.

Amplified products as in Example 6 were used as a probe in northern analysis. After visualising the amplified products on 1.5 % agarose gel these were cut from the gel and the DNA was eluted from the gel using QIAEX II gel extraction kit from M/s. Qiagen, Germany following the manufacturer's instructions.

Purified fragments were radiolabelleled with $\alpha[^{32}P]$dATP (4000 Ci/mmole) using HotPrime Kit from M/s. GenHunter Corporation, Nashville, USA following their instructions. Radio-labelled probe was purified using QIAquick nucleotide Removal Kit (QIAGEN, Germany) to remove unincorporated radionucleotide.

For blotting, 20 µg of RNA was run on 1.0 % formaldehyde agarose gel essentially as described in Example 1. Once the run was completed, gel was washed twice in DEPC trteated autoclaved water for 20 minutes each with shaking. Gel was then washed twice in 10 X SSPE (1.5 M sodium chloride, 115 mM NaH_2PO_4, 10 mM EDTA) for 20 minutes each with shaking. In the mean time nylone membrane (boehringer mannheim cat. no.# 1209272) was wetted in DEPC water and then soaked in 10 X SSPE (1.5 M sodium chloride, 115 mM NaH_2PO_4, 10 mM EDTA) for 5 minutes with gentle shaking. RNA from the gel was then vacuum-blotted (using pressure of 40 mbar) onto nylon membrane using DEPC-treated 10 X SSPE (1.5 M sodium chloride, 115 mM NaH_2PO_4, 10 mM EDTA) as a transfer medium. Transfer was carried out for 4 hours. Pressure was Increased to 70 mbar for 15 minutes before letting out the gel from the vacuum blotter.

After the transfer, gel was removed, and the location of RNA marker was marked on the nylon surface under a UV light source. Membrane was dried and baked at 80°C for 45 minutes. After a brief rinse in 5X SSPE (20 X SSPE: 3M sodium chloride, 230 mM sodium phosphate, 20 mM EDTA) membrane was dipped into prehybridization solution (50 % formamide, 0.75 M NaCl, 50 mM sodium phosphate, pH 7.4, 5 mM EDTA, 0.1% Ficoll-400, 0.1% BSA, 0.1% polyvinypyrollidone, 0.1% SDS solution and 150 ug/ml freshly boiled salmon sperm DNA) for 5 hours.

Radiolabelled probe synthesized earlier was denatured by boiling for 10 minutes followed by addition to the prehybridization solution dipping the blotted membrane. Hybridization was carried out for 16 hours. Solution was removed and the membrane was washed twice with 1X SSC (20X SSC; 3M sodium chloride and 0.3M sodium citrate dihydrate, pH, 7.0) containing 0.1% SDS at room temperature for 15 minutes each. Final washing was done at 50 °C using pre-warmed 0.25X SSC containing 0.1% SDS for 15 minutes. Membrane was removed, wrapped in saran wrap and exposed to X-ray film for 12-240 hours depending upon the intensity of the signal.

While performing northern hybridization, RNA from ND and D apical buds are blotted on the membrane and tested for the probe of choice. Figures 18-19 show the results with 4 such probe and confirm differential expression between ND and D apical buds.

31.2 (T11C, AP37) which is basically a 3' end region of the gene, hybridized to the transcripts 0.96 kb size on northern blot as in figure 19.

21.2 (T11C, AP7) which is basically a 3' end region of the gene, hybridized to the transcripts 0.96 kb size on northern blot as in figure 18.

53.1 (T11A, AP34) which is basically a 3' end region of the gene, hybridized to the transcripts 0.95 kb size on northern blot as in figure 19.

44.3 (T11G, AP33) which is basically a 3' end region of the gene, hybridized to the transcripts 1.75 kb size on northern blot as in figure 18.

Example 8

Method to correlate the identified gene with the dormancy of tea buds:

Above Example 6 identified 4 differentially expressed genes cloned by us. To further correlate these differentially expressed genes with the phenomenon of dormancy, the dormant bush during winter month was forced to break bud dormancy using a plant growth regulator gibberellic acid (hereinafter referred to GA_3). GA_3 was dissolved in 5% ethanol to yield a final concetration of 5 μM. The solution was applied onto each dormant bud with the help of a paint brush at least 4 times on the same day during the month of December. Bud length and its perimeter was recorded regularly as an indicator of its growth. Out of 20 buds only 10 buds showed increase in growth (dormancy break) in response to GA_3 application. These were termed as forced ND apical buds. These were collected and stored as mentioned in Example 1 and used for the purpose of RNA isolation to be used in northern analysis.

For such an experiment, RNA from ND, D and forced ND was blotted onto a nylon membrane and probed with ND (31.2) probe probe. Various procedures involved are already mentioned in Example 6.

As can be seen from Figure 20 that the expression of the gene for the probe was down-regulated (i.e. showed lesser expression) in D apical buds compared to the ND. Again the gene showed up-regulation (i.e. showed over-expression) in the forced ND. Thus, it proved that the gene 31.2 (sequence ID No. 1, in the present invention as detailed in Example 8) is indeed a gene related to dormancy. Similar approach can be adopted for other genes as well.

Example 9

Sequencing the identified clones:

Each clone was sequenced manually using a T7 sequenase version 2 sequencing kit from M/s. Amersham Pharmacia Biotech, USA. Sequencing primers used were [Lgh (5'-CGACAACACCGATAATC-3') or Rgh (5'-GACGCGAACGAAGCAAC-3')]

(1) INFORMATION FOR SEQ ID NO: 1
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 305base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular
(ii) MOLECULE TYPE: cDNA
(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 1
Seq ID No. 1# Sequence of 31.2 clone:

5'- ATCGCCGTAA TTGCCATGTT TTCCCTCTCA CCGGAATCCT ACG
TTATCC CCTTACCTTC GTGAACATTA CAGTAGGAAT CGGTGGTCCA
ATTATCAACT TAATTTTGGG CGCATCTGTT CGTGTTAACT AGAAGCCATG
TATACATACA ATACAACATG GTTCACTCCT CCTACAGATT ATGAGTTGAA
CTTTTATAAT AAGTTGTAAT AATGGCTTCT GAATAAGGAG AAGAGGAGCC
TCTGTTTGTT TTACTTATTA CAGATGTGAT ATCGTTCAAC AACTTTGATT
CTGCGAAAAA AAAAA-3'

(2) INFORMATION FOR SEQ ID NO:2
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 297 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular
(ii) MOLECULE TYPE: cDNA
(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 2

Seq ID No.2# Sequence of 21.2 Clone:

5'- AGAAGTACCT GAAAGGAAGC TTAACGAGGT GAACATCCAT TGCAGCCAGC CCTGGAATCT GTACAGGGCA ACTCTGAACC GGAATTATTT TAATAACCCG TGGGCAATGA TTGCAATTAT GGCTCGTTTG GTATTACTTC TACTCACTTA GACACAACTG TATTTACGGT TTTCGCTGGA ATTGTAATTG TTGGAGCGAC AAAATAGATG GTCACAACTT ATTGGTGAGA GTATCAGTGT GCTCTTCTTT ATCGTCTTTA ACTCTCCGTG GTAATTACTT TGACAATATT CATACAT -3'

(3) INFORMATION FOR SEQ ID NO: 3
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular
(ii) MOLECULE TYPE: cDNA
(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 3
Sequence ID No.3# 53.1 Clone:

5'- GAGACTCAGC TCAGCAATCA TGTTCTAAGT GAATGTCACT CTATCGCCTT CTTGTCCCTC TTAGACATAC TACATCCTCA TTCTGCTAGA AATGAACTCA TGTAGGTTTT GAAGTTGGGA ACTTTTGAAA CTGTGTTGTT TGGGTGCTGT CTGTTATACA ATTCTCTCAA CTGCGGAGAA TTGACGTTGG TTGTAGTGGA ATTCAACACT TGGGTTTTGT TCTTAGTTAA AAAAAAAAA-3'

(4) INFORMATION FOR SEQ ID NO: 4
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 228 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular
(ii) MOLECULE TYPE: cDNA
(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 4
Seq ID No.4# Sequence of 44.3 Clone:

5'-ATAGCTTAGT CACGTGTCTC TTGAGAATGG ACTACGTAGT TGTTAAGTTG GGTGATCAGA AGGCGTTGAT GATGAATGTA TGAAGCAGAG ACTACTGAAT GTAATTTTGT TGTTGAAAGA TGAATGATTT ATTAATGCCT GCATATCTTT CTATTGTTTG ATGCCAAACC TTTGGGCACA TTTTTTCTTT CTTTTTGTGA TAATGTTCTC TTCTTGCAAA AAAAAAAA-3'

Example 10

Analysis of the sequences:

Each clone was subjected to BLAST analysis and the clones were found to be unique.

The main advantage of the present invention are:

(a) Novel DNA sequences expressed and repressed during winter dormancy in the apical buds of *Camellia sinensis* L. (O.) Kuntze (tea) bush under field conditions have been cloned

(b) These sequences have been cloned from the same genetic make up of the tea bush

(c) A spectrum of 3' ends of the expressed and repressed genes in non-dormant and dormant apical buds of tea bush growing under field conditions has been presented

(d) Confirmation of the identified 3' ends of the differentially expressed gene(s) for establishing differential expression during winter dormancy in the apical buds of tea bush growing under field conditions has been carried out.

(e) Method to correlate the identified gene with the dormancy of tea buds has been described under field conditions

(f) Sequencing of the cloned 3' ends of the differentially expressed gene(s) showed uniqueness in terms of novel sequences not deposited in the data bank so far